

The role of RNA silencing in *Tospovirus* infection and transgenic resistance

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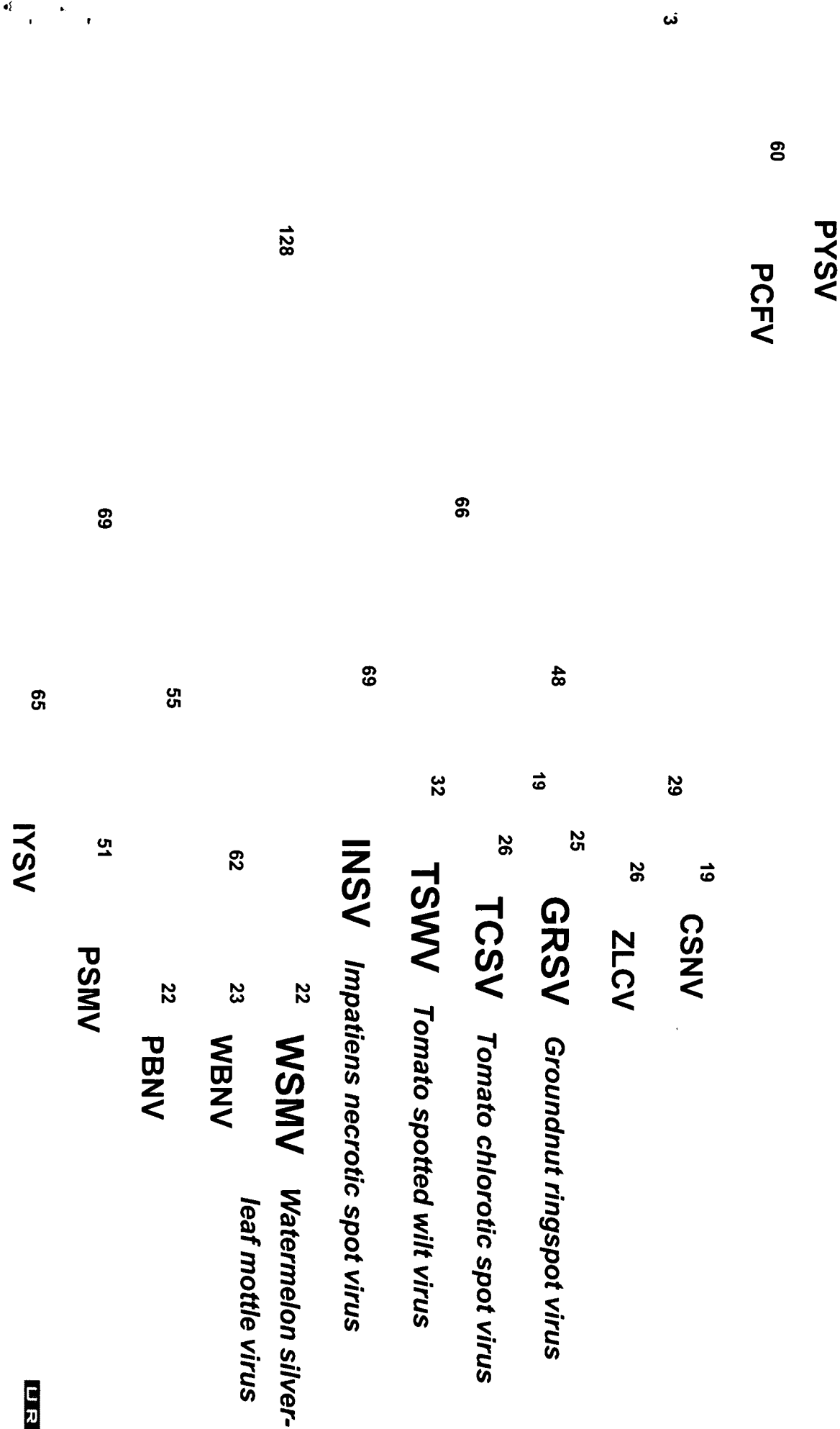
TOSPOVIRUSES (family *Bunyaviridae*)

Type species tomato spotted wilt virus (TSWV)

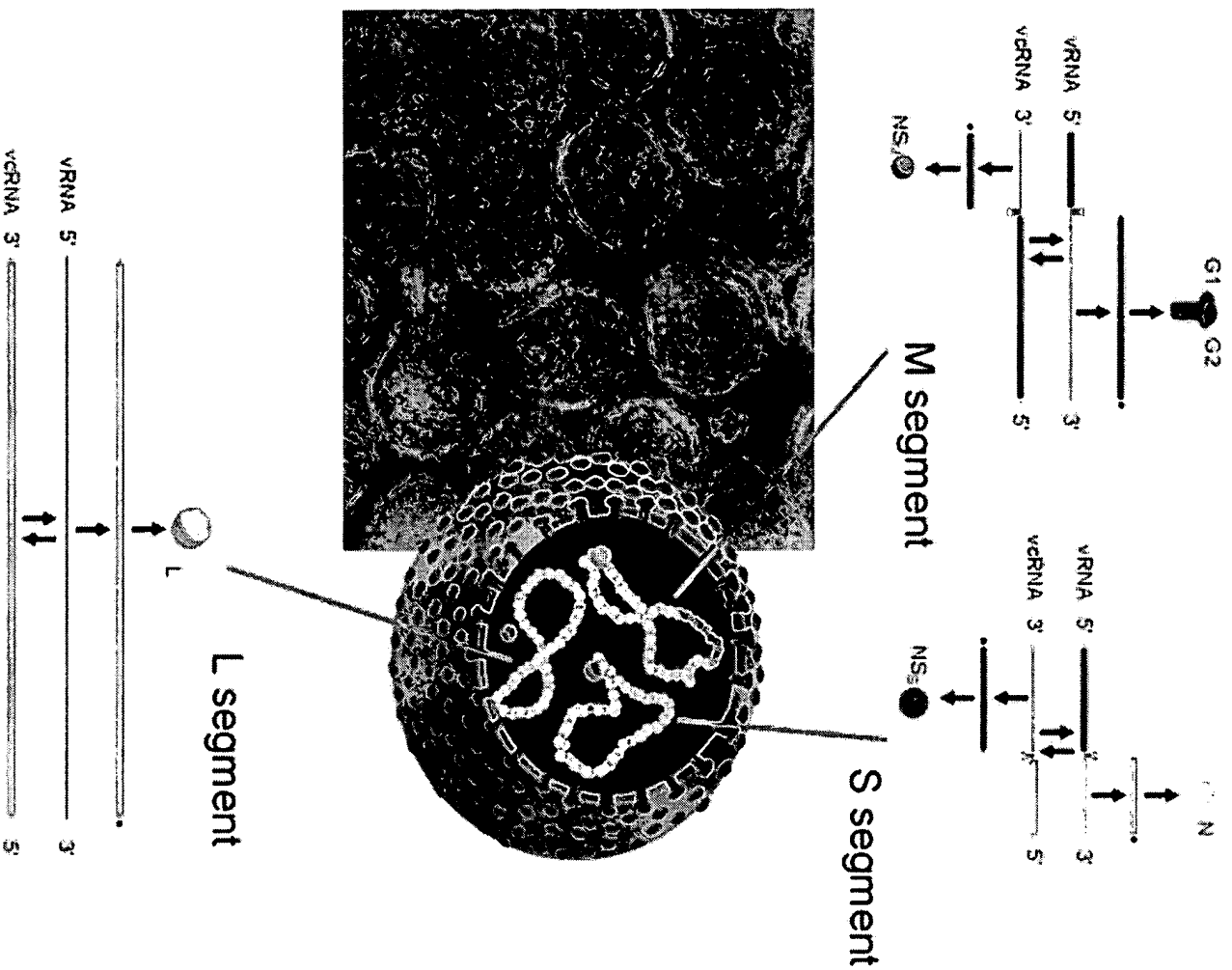
- ➔ **More than 10 established species**
- ➔ **Transmitted by thrips in a propagative manner**
- ➔ **Causal agents of major diseases in many economically important crops and ornamentals**



Tospovirus family



TSWV



The five genes of TSWV:

L polymerase

NS_M movement protein

G1/G2 glycoproteins

N nucleocapsid

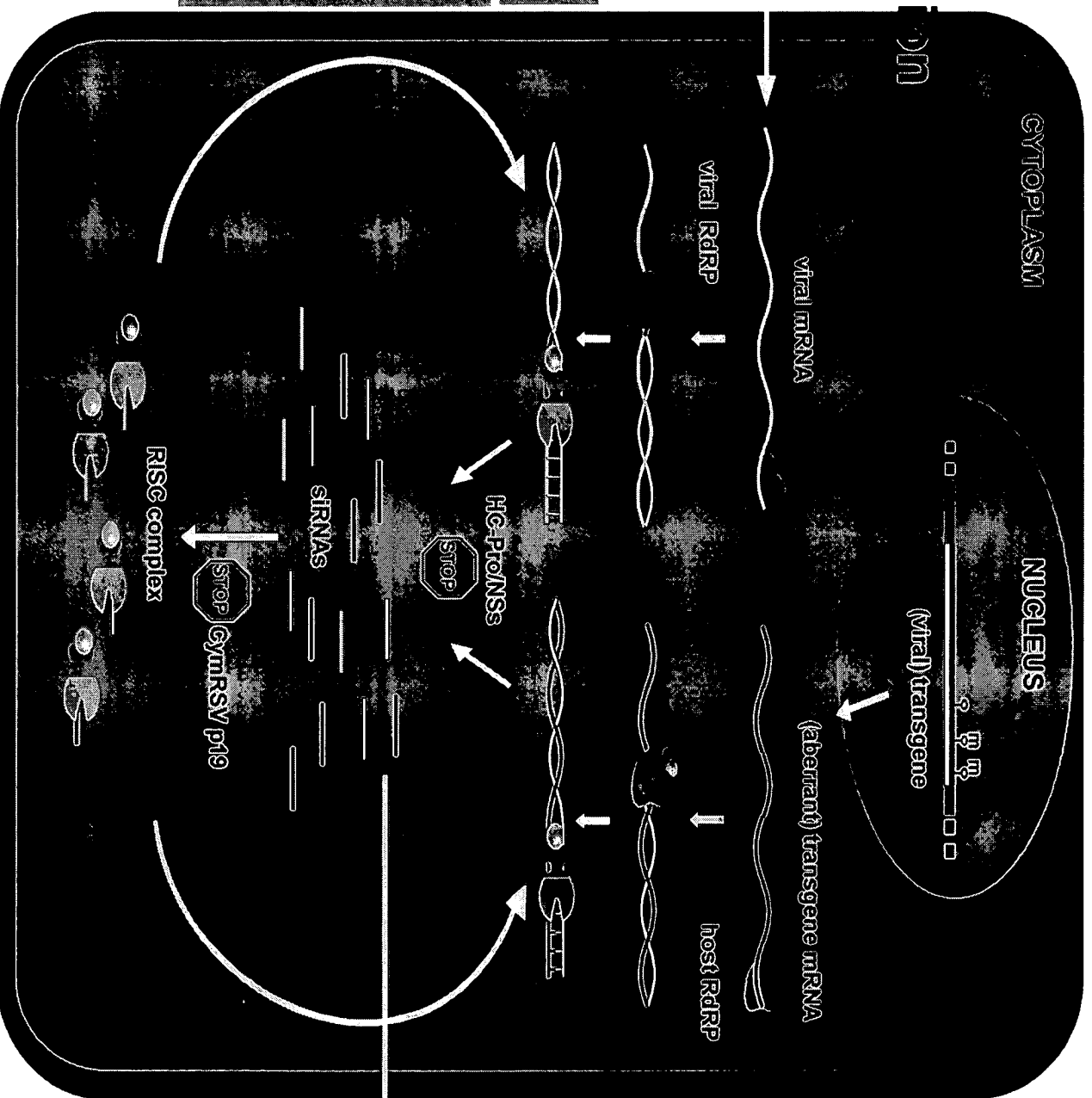
NS_S silencing suppressor



**DTGs for virus
resistance /
silencing suppression**

invading virus

	allana	C. elegans	assa	D. melanogaster
SGS3				
p SDE1 SGS2 QDE1 EGO1				
ase Dicer DCR Carpelfactory				
helicase SDE3				
Argonaute SGS4 AGO1 RDE QDE2				



PYX p25

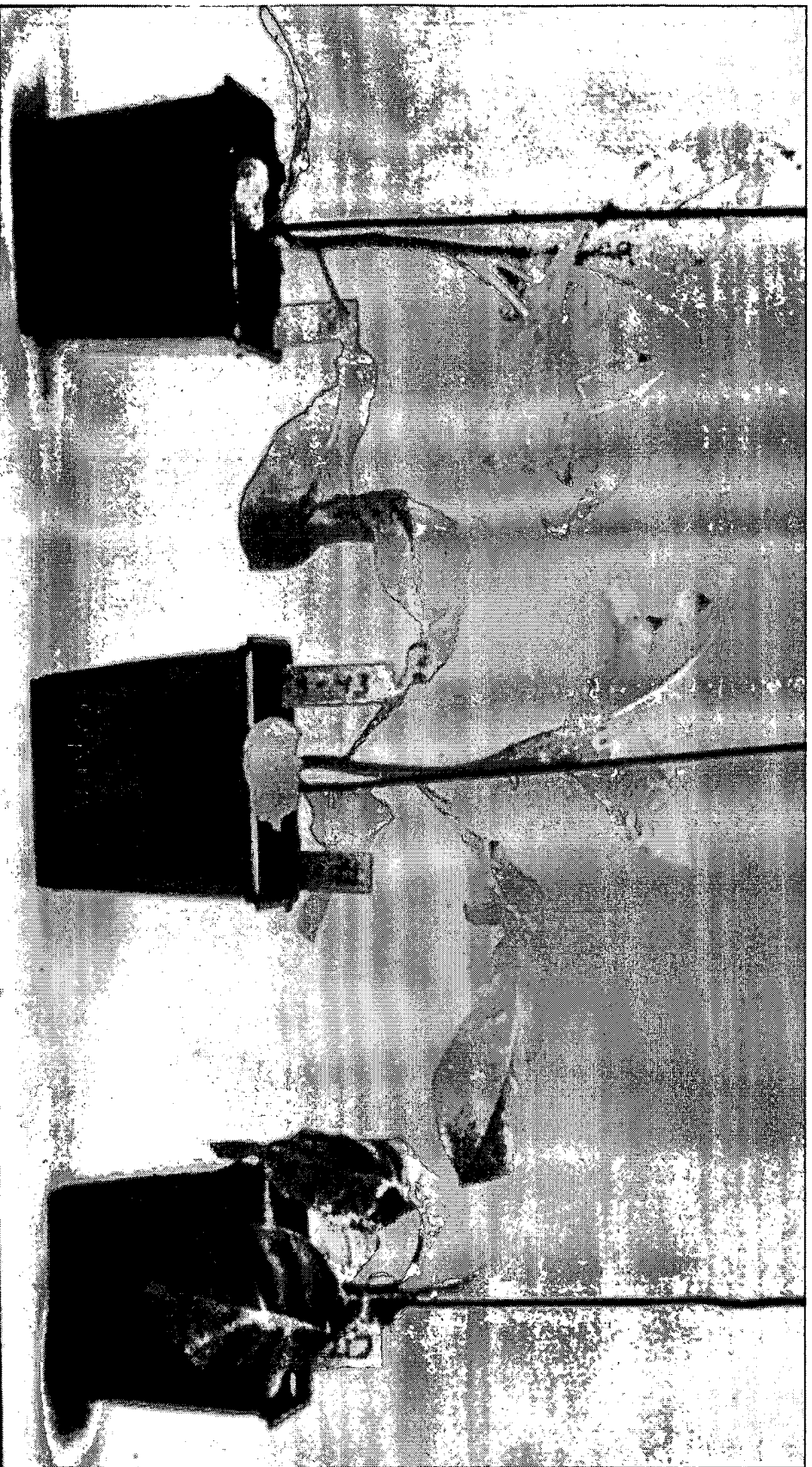
STOP

systemic sign:



‘The old days’

**RNA-mediated resistance; Plant Viruses, 1991
Post Transcriptional Gene Silencing (PTGS)**



(De Haan et al, 1991)

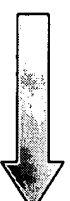
Post transcriptional gene silencing (PTGS)

- **Characterized by high transcription rates and a low steady-state RNA level**
 - **Posttranscriptional degradation in the cytoplasm**
- **Resistance is RNA-mediated (untranslatable mRNAs also confer resistance)**
- **Silencing is very sequence specific**
 - **Resistance is narrow**
- **dsRNA triggers silencing**



Transgenic resistance by PTGS

- Transgenic resistance can be obtained by the transformation of a (partial) viral gene into a plant
- Single gene silencing
 - <<10% of transformed plants
- Repeated sequences: inverted repeat
 - 60%
- Intron(-spliced) inverted repeats
 - 90%



Virus resistance against CMV

Constructs

Resistance in R_0



0% (11% in S_1)

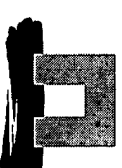


75%

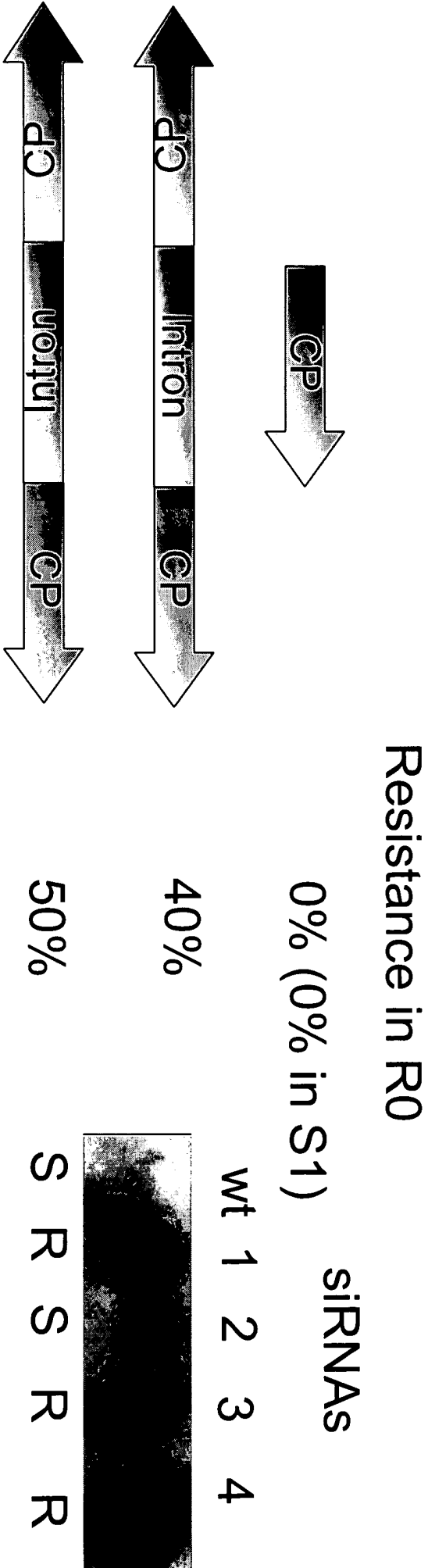
siRNAs

wt 1 2 3

33%



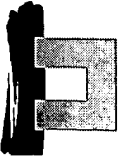
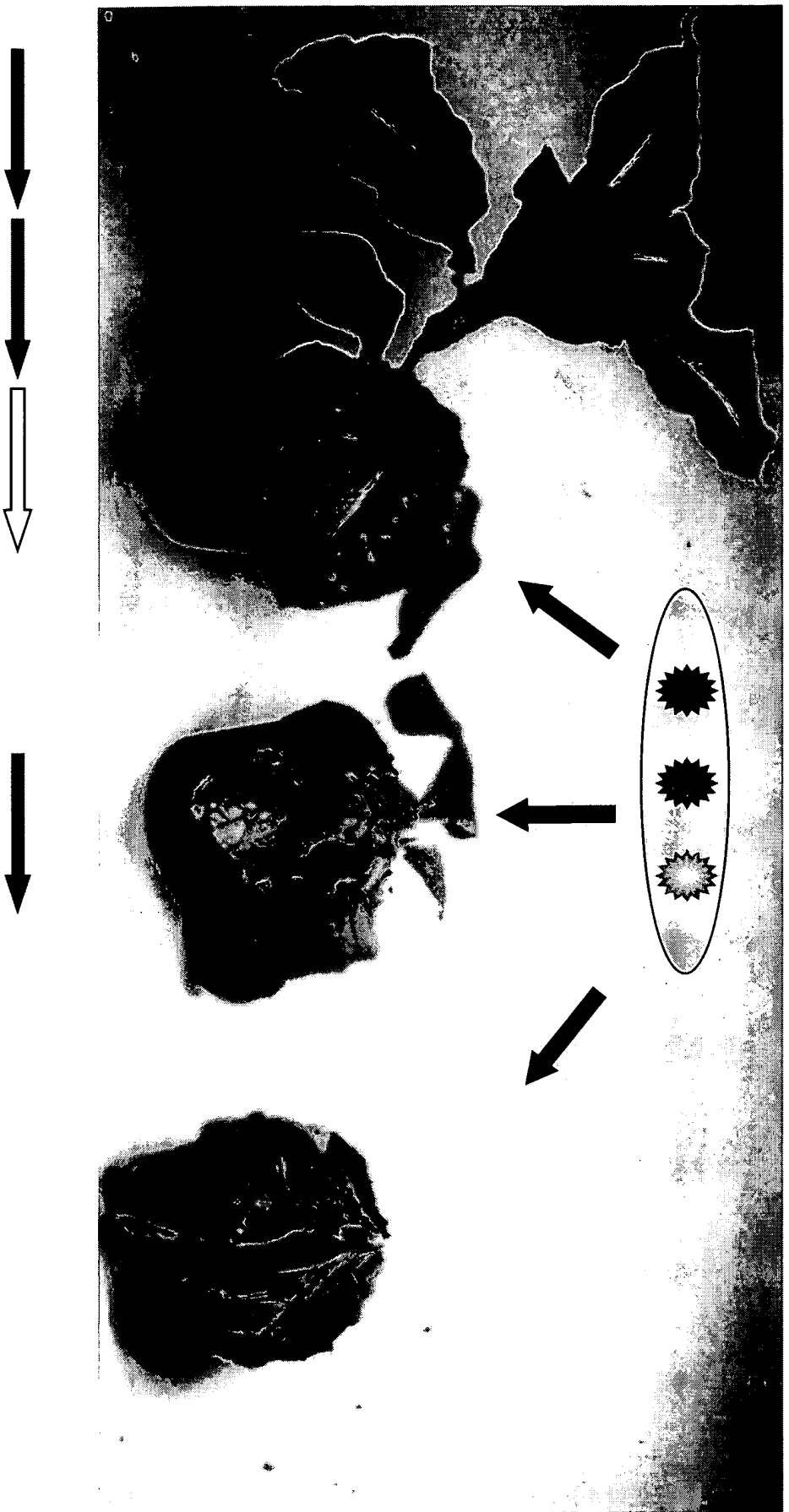
Virus resistance against CMV



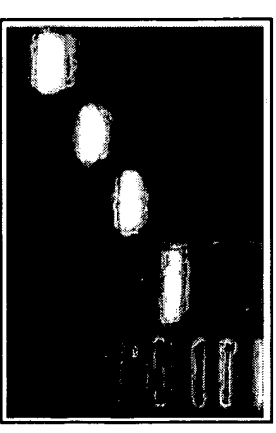
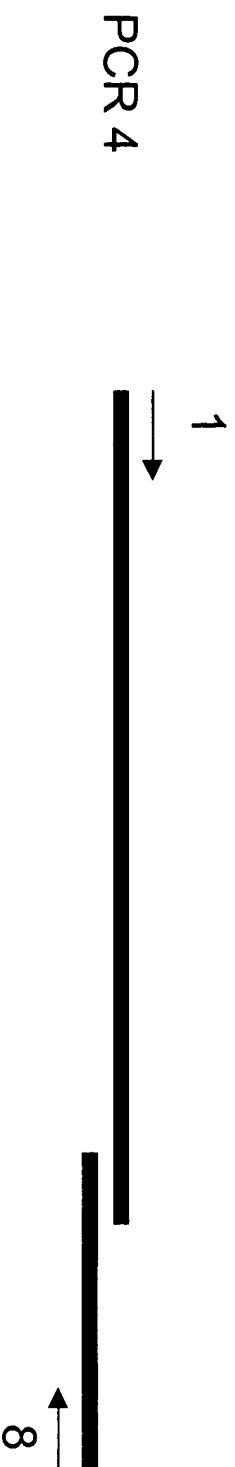
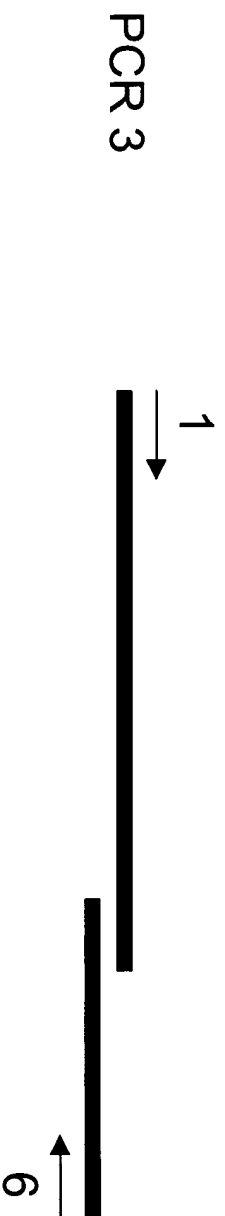
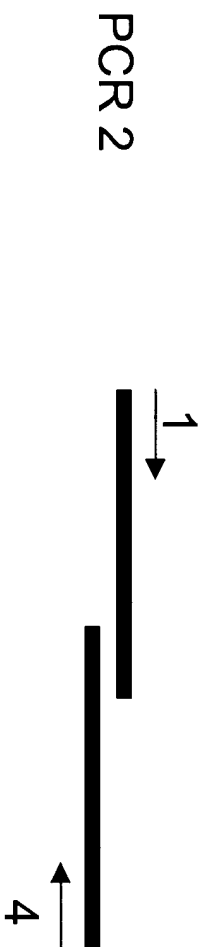
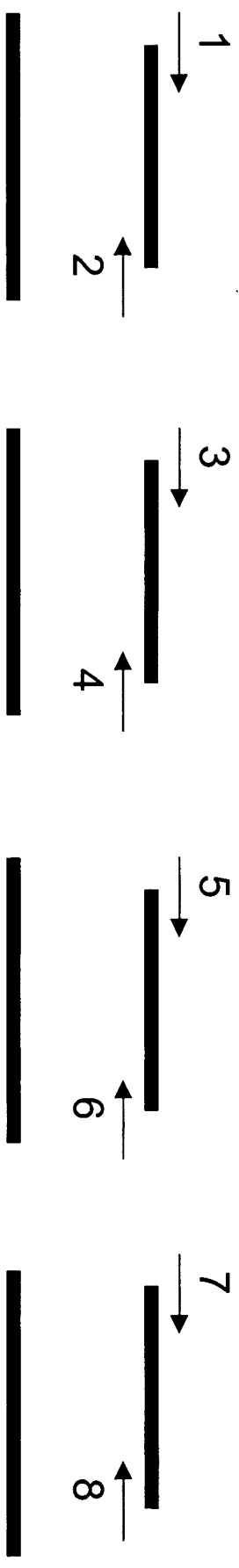
Resistance in the IR S1 lines was always 100%



Broad tospovirus resistance using multiple transgenes

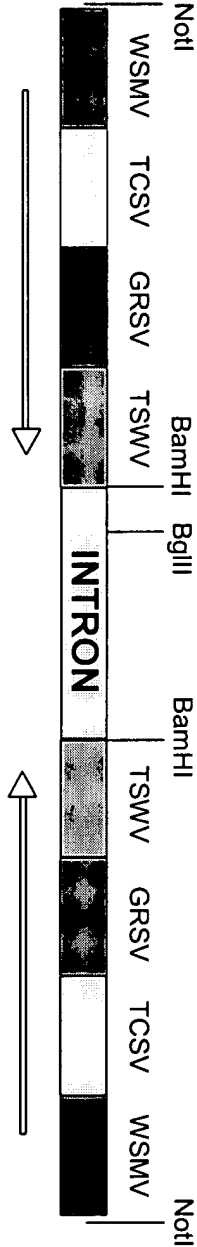


Using fusion PCR to create a multi-tospo construct

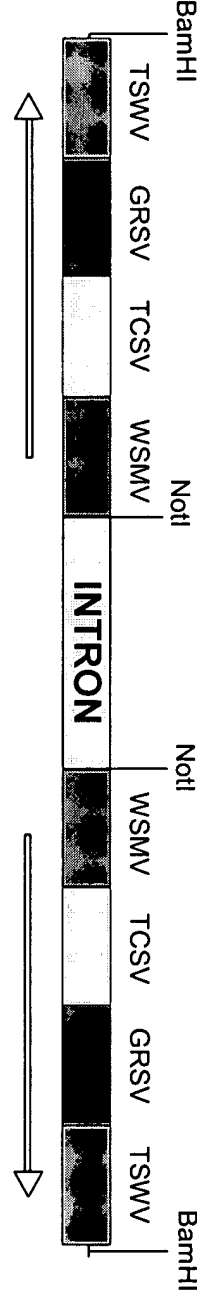


Tospoviral inverted repeat cassettes

IRN
SA

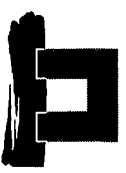
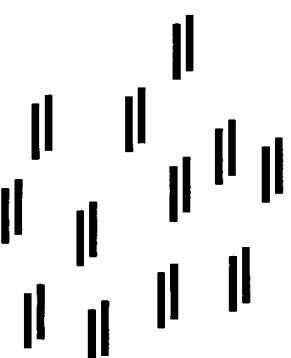
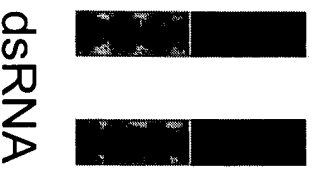
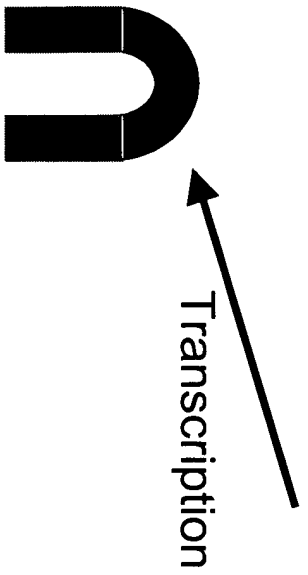
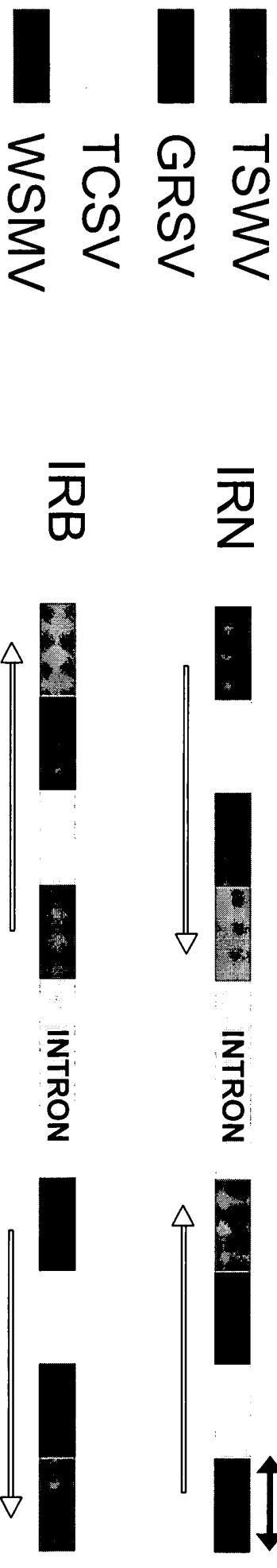


IRB
AS



Tospoviral inverted repeat cassettes

N-gene segments:



Resistance analysis of the IRB plants



Line name	TSWV	GRSV	TCSV	WSMV	Mix
IRB2	100%	N/A	N/A	N/A	N/A
IRB4	100%	100%	100%	100%	100%
IRB6	100%	100%	100%	100%	100%
IRB7	100%	100%	100%	100%	100%
IRB8	100%	100%	100%	100%	100%
IRB9	100%	100%	100%	100%	100%
IRB10	100%	100%	100%	100%	100%
IRB11	100%	100%	100%	100%	100%
IRB12	100%	100%	100%	100%	100%
IRB14	100%	100%	100%	100%	100%
IRB15	100%	100%	100%	100%	100%
IRB16	100%	100%	100%	100%	100%
IRB19	100%	100%	100%	100%	100%
IRB20	100%	100%	100%	100%	100%

82% of all IRB lines are resistant against all four tospoviruses



Resistance analysis: an example

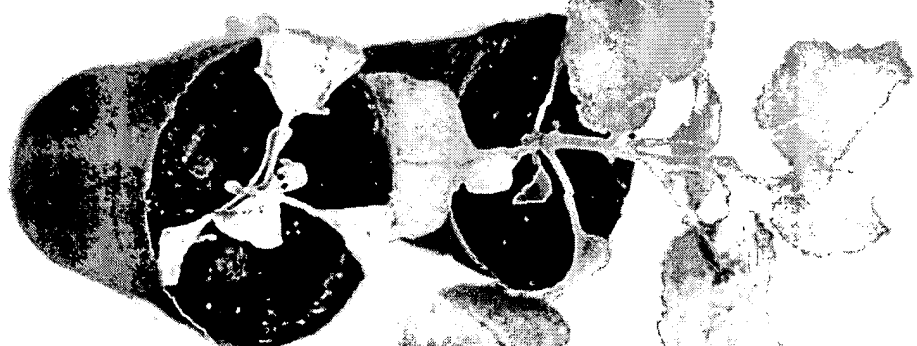
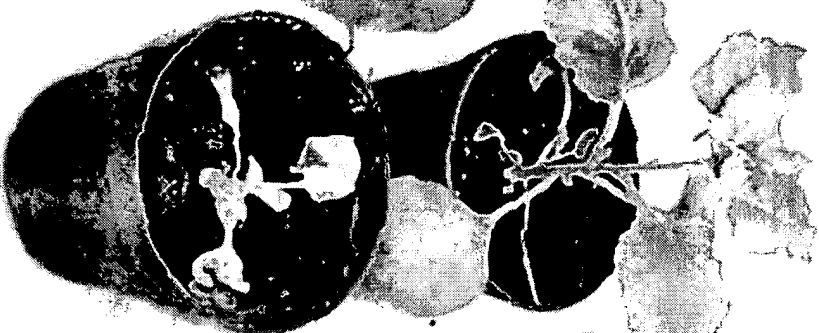
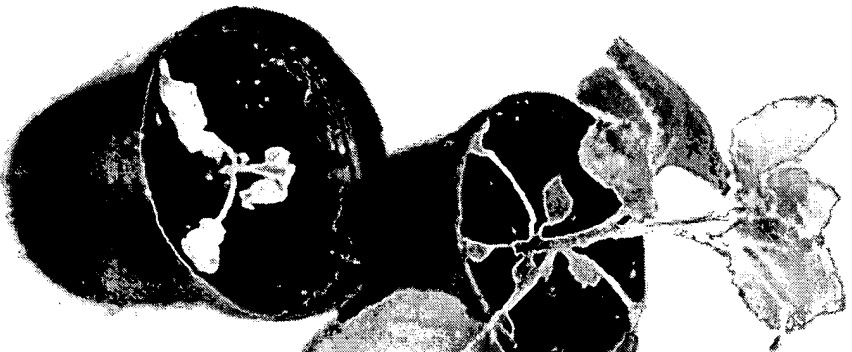
TSWV

GRSV

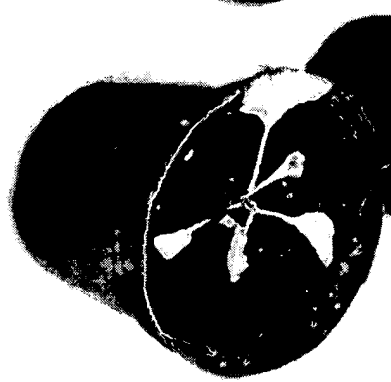
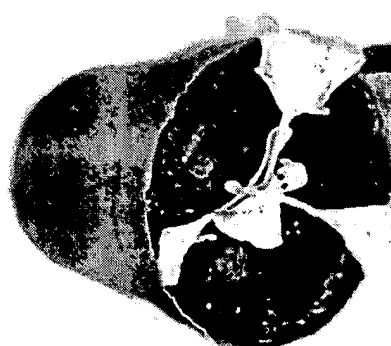
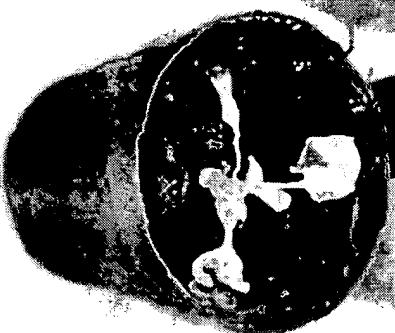
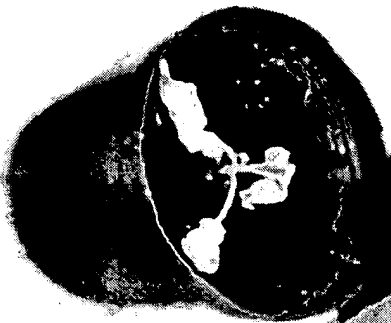
TCSV

WSMV

IRB16



IRB17



Resistance analysis of the IRN plants

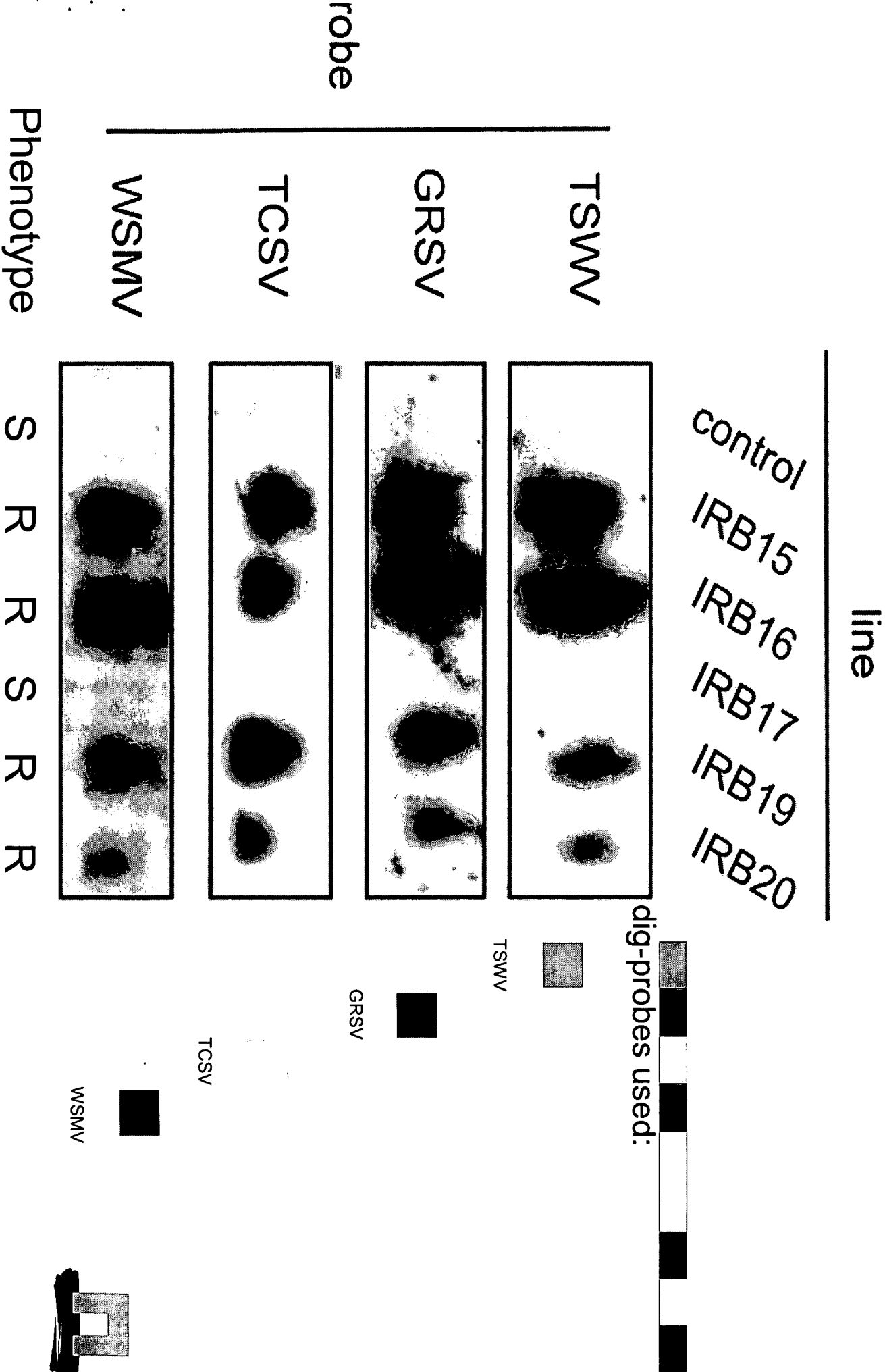


Line name	TSWV	GRSV	TCSV	WSMV	Mix
IRN6	66%	100%	100%	100%	100%
IRN8	100%	100%	100%	100%	100%
IRN9	100%	100%	100%	100%	100%
IRN10	100%	100%	100%	100%	100%
IRN14	66%	100%	100%	100%	100%
IRN15	100%	100%	100%	100%	100%
IRN19	100%	100%	100%	100%	66%
IRN20	100%	100%	100%	100%	66%
IRN21	0%	0%	0%	100%	0%

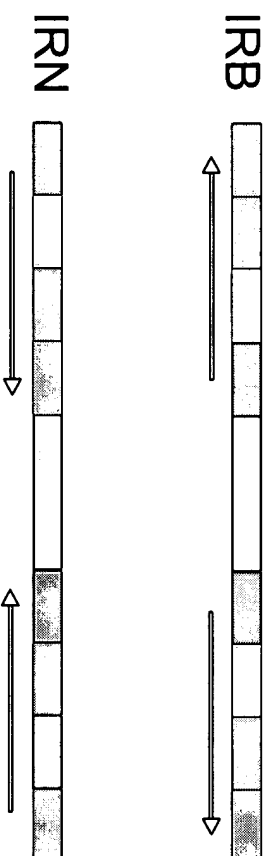
58% of all IRN lines were resistant against all four tospoviruses



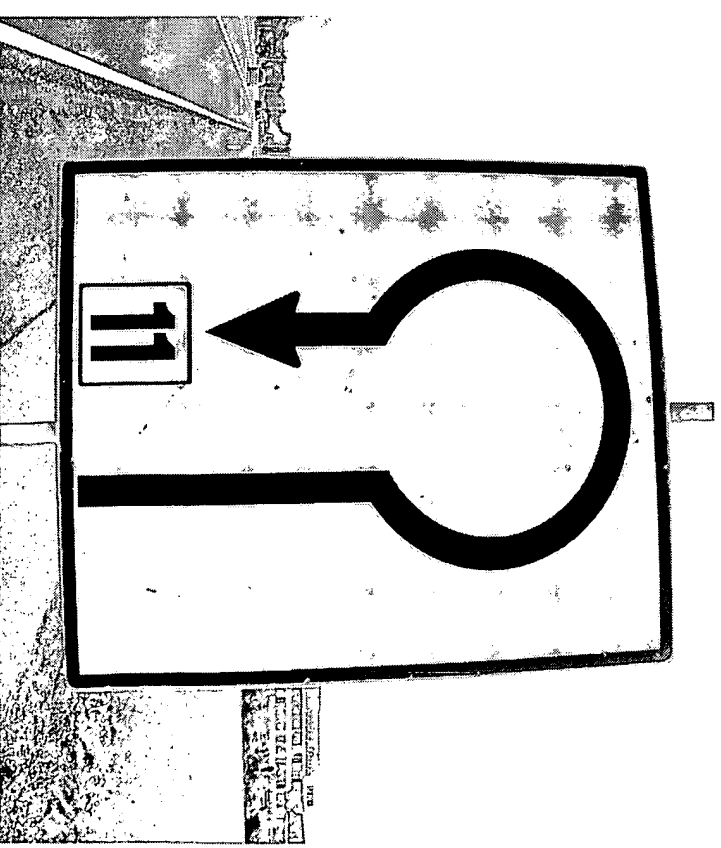
Molecular analysis of several IRB lines: transgenic 'viral' siRNAs



Resistance analysis

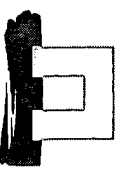


High levels of multiple virus resistance

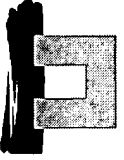
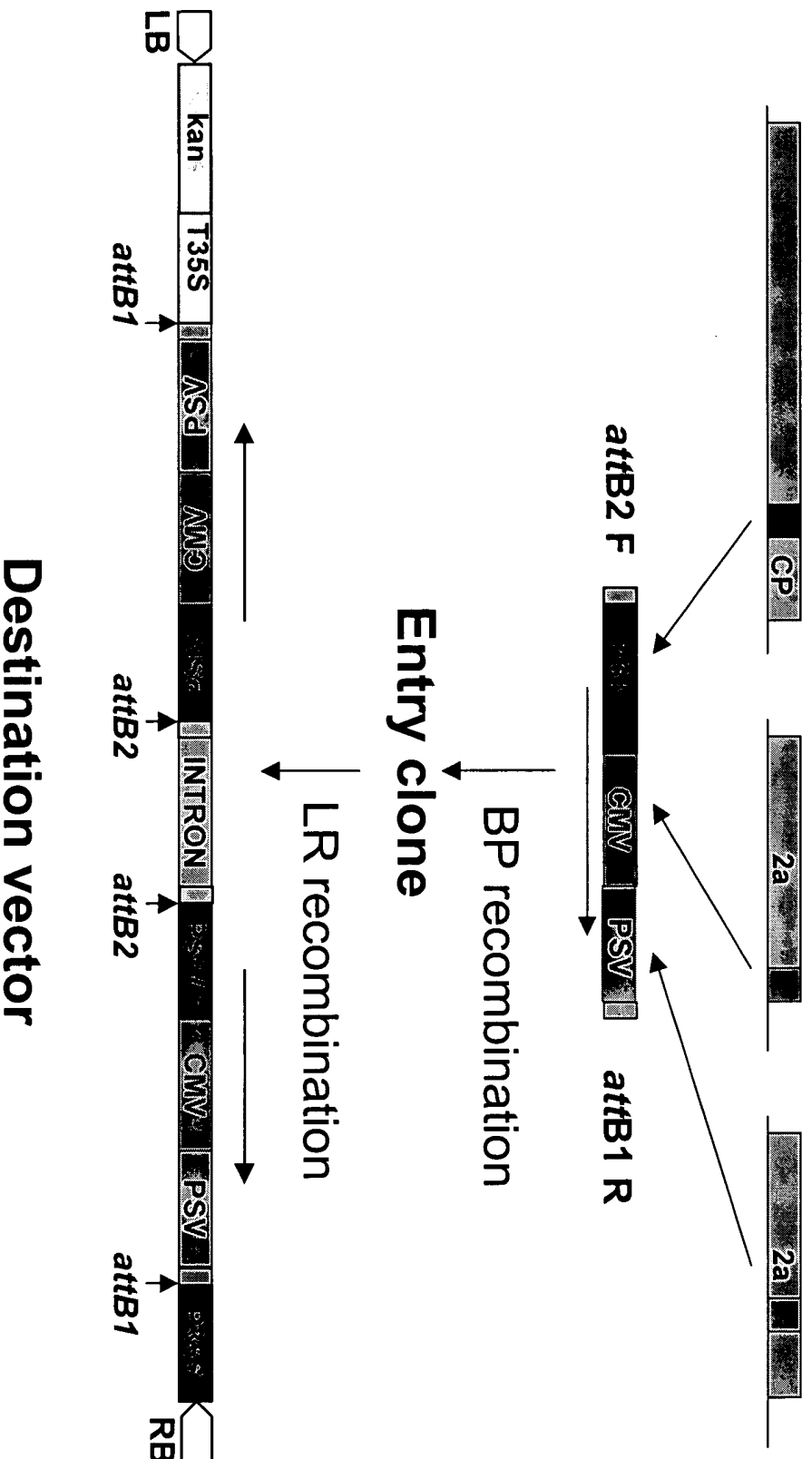


Resistance in difficult-to-transform plant feasible

Beware of silencing suppressors



Strategy to fuse different fragments of peanut viruses and transfer into inverted an IR binary vector using Gateway



Conclusions

TSWV and CMV can be stopped by enhanced gene silencing (transgenic resistance)

IR constructs are very powerful inducers of gene silencing

Stretches as short as 150 nt are sufficient for resistance

Linking viral sequences results in all-or-nothing resistance: resistance to one virus = resistance to all viruses

Very high resistance frequencies (>80% R_0) make easy transformation possible; even of 'difficult crops'

